CULTIVATION AND SPORULATION OF BACILLUS SPECIES IN SELF HEALING CONCRETE: A SYSTEMATIC LITERATURE REVIEW

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SCHOOL OF CIVIL ENGINEERING
UNIVERSITI SAINS MALAYSIA
2021

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by

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This dissertation is submitted to

UNIVERSITI SAINS MALAYSIA

As partial fulfilment of requirement for the degree of

BACHELOR OF ENGINEERING (HONS.)
(CIVIL ENGINEERING)

School of Civil Engineering
Universiti Sains Malaysia

Jul 2021

SCHOOL OF CIVIL ENGINEERING
ACADEMIC SESSION 2020/2021

FINAL YEAR PROJECT EAA492/6
DISSERTATION ENDORSEMENT FORM

Title: CULTIVATION AND SPORULATION OF BACILLUS SPECIES IN SELF HEALING CONCRETE: A SYSTEMATIC LITERATURE REVIEW

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Date:

ACKNOWLEDGEMENT

History of all great work into witness that no great work was ever done without either active or passive support of person ‘surrounding and one’s close quarters. Thun is it not hard to conclude how active assistance from senior could passively impact the execution of a project. I am highly thankful to my supervisor, Professor.Ir.Dr.Badorul Hisham Abu Bakar for his active guidance throughout the completion of project.

Last but not least, I would also want to extend my appreciation to those who could not be mentioned here but have well played their role to inspire me behind the certain.

ABTRAK

Kerja pembaikan dan pemulihan konkrit hampir tidak dapat dielakkan dalam kebanyakan struktur konkrit. Rawatan menngunakan bakteria (spesies *Bacillus*) banyak digunakan dalam konkrit lestari (konkrit penyembuhan diri) untuk memberikan kos pembaikan yang lebih cekap dan teratur dari segi keseluruhan strukturnya. Untuk membolehkan konkrit penyembuhan diri dikomersialkan, media digunakan untuk menghasilkan spesies mikroba perlu ditangani dengan lebih cekap. Tujuan utama tinjauan literatur ini adalah untuk memberikan dapatan terkini mengenai kaedah pembiakan sporulasi spesies *Bacillus* dalam konkrit penyembuhan diri dan cara mengenalpasti prestasinya dalam kuantiti yang optimum. Tinjauan ini dirancang mengikut tatacara Item Pelaporan Pilihan untuk Kajian Sistematik dan Meta-analisis (PRISMA) bagi mengelakkan sebarang ketidakseimbangan pemilihan data dan meningkatkan kebolehpercayaan data yang diekstrak dan disintesis. Selanjutnya, terdapat sejumlah 11 makalah yang berkaitan dengan bahan tambahan lain melalui rujukan silang. Semua data kertas ini diambil berdasarkan informasi umum, bakteria, persediaan eksperimen, penilaian penyembuhan, dan hasil eksperimen. Walaupun bakteria diterapkan dalam setiap eksperimen, respons terhadap faktor tersebut menunjukkan dapatan yang hampir sama dari segi pH, bekalan oksigen, suhu, medium kultur, dan nutrien. Medium yang sesuai sangat penting dalam setiap tahap (pertumbuhan, pembiakan, pemendakan), bagi memaksimumkan prestasi konkrit penyembuhan diri. Hanya dengan teknik penilaian yang sesuai, seorang pereka dapat merumuskan resipi konkrit penyembuhan diri mikrob yang optimum. Walau bagaimanapun, ada lebih banyak kemungkinan untuk merumuskan konkrit penyembuhan diri mikroba yang tidak terkawal hanya untuk spesies Bacillus. Tinjauan ini mengikuti penyelidikan 5 tahun terakhir (2016-

2020) di bidang kejuruteraan namun terdapat lebih banyak spesies mikroba berpotensi masih belum dapat dijumpai di masa depan.

ABSTRACT

Concrete repair and remediation work almost inevitable in most concrete structures. Active treatment is the solution which is bacteria (Bacillus species), widely adopted in the new sustainable concrete (self-healing concrete) to provide a more efficient and better life cost in terms of its overall structure. To allow self-healing concrete to be more commercialized, the factor, media used to produce the microbial species need to be precisely addressed accordingly. The main aims of this review are to provide information regarding the method of cultivation and sporulation of Bacillus species in self-healing concrete and the method to identify its performance to obtain the optimum condition. This review is planned according to (Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) to prevent any unbiased and increase the reliability of the data extracted and synthesis. Furthermore, there are a total of 11 papers included in this review associate with other supplementary material through cross-referencing. All of these paper data were extracted based on its general information, bacteria, experimental setup, evaluation of healing, and experimental result. Although bacteria strains are applied in each experiment, its response to the factor showed almost similar results in terms of pH, oxygen supply, temperature, culture medium, and nutrient. A suitable medium is crucial in every stage (growth, reproduction, precipitation), as it can maximize the performance of the self-healing concrete. Only with suitable evaluation techniques, a designer can formulate an optimized microbial self-healing concrete recipe. However, there are more possibilities of formulating microbial self-healing concrete which is not restrained to Bacillus species only. This review followed the recent last 5 years of research (2016-2020) on the engineering field, more other potential microbial species are yet to be discovered in the future.

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CHAPTER 1

INTRODUCTION

1.1 Background

Cement is the most vital element in the concrete material as it binds the aggregate and fills up the void between the coarse and fine particles. In any application, concrete load-carrying capacity usually refers to its compressibility. In other words, the concrete able to carry the compressive load safely and provide structural functionality with negligible deflection. However, the brittleness of concrete which leads to crack offsets the integrity and bearing capacity of the structure (Zhang et al., 2020).

Theoretically, cracks that have a small opening size (<0.2mm) are considered a non-problematic surface defect and do not influence the strength, performance of the structural properties (Farhadi and Ziadloo, 2020). In certain circumstances, the cracks that developed earlier due to creep shrinkage or overstressed will allow the aggressive substance and moisture to penetrate (Hizami Abdullah et al., 2018). This will initiate the carbonation in concrete and corrosion to the reinforcement that embedded within the concrete.

As above mentioned, the crack will influence the structural integrity, thus effective repair and maintenance are crucial to extend the structure service life. Conventional repair and remediation work are mostly restricted to small-scale maintenance, as it will be very costly for large infrastructures due to the complexity and practicability of remediation work (Zhang et al., 2020). On the other hand, repairing work which consists of refurbishment, demolition, and maintenance requires a huge amount of material and severe impact to biodiversity due to high energy demand and excessive mineral exploitation. Although concrete has a lower value energy input in terms of emission per unit mass when compared to other structural materials for infrastructures such as steel and timber, the high-rate consumption still reveals total high energy consumption and carbon emission (Sangadji, 2017).

Several interventions have been made to tackle the issues above mentioned to ensure the sustainability of the structure in terms of material usage. Flatt et al suggested that this can be done by extending the service life of existing infrastructures (Flatt et al., 2012). Therefore, the termination of crack at an early age is very critical. Generally, there are two types of crack treatment which are active and passive (Seifan et al., 2016a). Passive treatment only able to seal the surface crack by applying the coating such as chemical or admixture to the concrete surface whereas active treatment can heal both internal and external crack for enhancing the durability and preventing the penetration of aggressive agent outside and within the concrete (Wang et al., 2012;Pacheco-Torgal and Labrincha, 2013). Due to several limitations of passive treatment such as poor bonding at the interface between the sealant and the existing concrete surface, many of the researchers have switched their directions more to active treatment.

Active treatment usually refers to the self-healing techniques which can seal the crack independently regardless of the crack position (Seifan et al., 2016a). Self-healing techniques were first discovered in 1836 by the French Academy of Science in the form of autogenous self-healing (Zhang et al., 2020). Concrete healing is a natural phenomenon that can be illustrated by the human recovery system. The crack that forms on the concrete due to weathering, represents the wound causes on human skin. It can be recovered just like our body system, provided with sufficient healing elements inside the body itself. This phenomenon similar to concrete, not all the cement constituents will be utilized after the concrete gain its strength, some of the left cement particles can rejuvenate the strength in a condition that sufficient water is allowed for the cementation reaction. Basically self-healing techniques can be categorised into two major class which are autogenous and autonomous self-healing (Mihashi and Nishiwaki, 2012). In autogenous self-healing, the crack sealing mechanism is initiated without any external operation, whereas in autonomous self-healing, some engineered admixture is required to trigger the crack repair action (Sidiq et al., 2019).

Few researchers prove autogenous self-healing is more dependent on environmental conditions such as crack width and water availability. Moreover, the environmental element is unlikely to be controlled and difficult to be measure due to its variability, it is hard to quantify the healing mechanism of a structure over a certain period (Sangadji, 2017). In fact, this type of healing can only recover small crack width up to 200μm only (Ter Heide et al., 2005;Schlangen et al., 2006;Van Tittelboom, 2012). In contrast, autonomous self-healing can be easily manipulated as all the engineered additives mixed in the cementitious matrix have their characteristics, thus the design parameter based on the specification are more practical to be achieved.

Recently, there are few researchers have developed and found interest regarding microbial self-healing due to its potential in environmental crack healing, efficient and long-lasting capability in recover concrete crack (Seifan et al., 2016b;Wang et al., 2016). In fact, microbial self-healing concrete often relates with Microbial Induced Calcium Carbonate Precipitation (MICP). Calcium carbonate precipitation can be in three forms of morphology such as calcite (rhombohedra crystal), aragonite or vaterite (hexagonal crystal) (Seifan et al., 2016a). Microbial self-healing will be activated from the stage of the bacteria hibernation period, with the aid of metabolic bacterial activities, calcium carbonate will be formed and reduce the gap caused by the crack (Vijay et al., 2017) .

There are two categories of bacteria are used in microbial self-healing which are the ureolytic strains and non-ureolytic (also known as alkaliphilic). Each of these bacterial strains has its own pathways to precipitate calcium carbonate.

1.1.1 Urea hydrolysis

Alkaline tolerant ureolytic strains secrete an enzyme named urease which act as a catalyst to hydrolyzed urea to ammonium and carbonate ions (equation 1.1), thus pH increases in the medium (Wang et al., 2016). For equation 1.2, the process takes place near the cell wall of the bacteria (Figure 1.1), highly concentrated negatively charge of carbonate ions attract calcium ions (from unhydrated cement particles or nutrient source) which merge and form calcium carbonate (Chaurasia et al., 2019). The most probable process reactions are shown as follows (Wang et al., 2016):

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4+ +CO_3^{2-} \tag{1.1}$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \tag{1.2}$$

The production of ammonium ion from the urease could lead to the formation of ammonium salt which promotes the corrosion of the reinforcement when a crack occurs. Moreover, the ammonia produced also could cause negative effects to the environment and human health, where this restricts the application in concrete structure (Jonkers et al., 2010). To counter this issue, a researcher had proposed several solutions such as nitrification by converting ammonia into nitric acid (Dhami et al., 2012) or using non ureolytic bacteria for MICP.

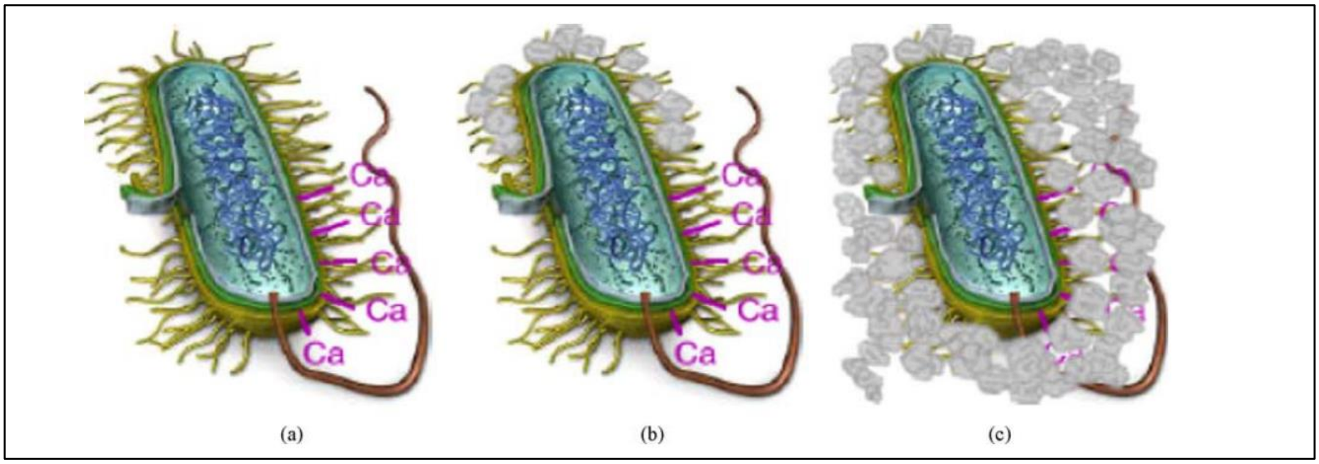

Figure 1.1 Calcium carbonate precipitation at cell wall. (a) Illustrates consumption of the CO_3^{2-} source by the bacterium , and secretion of dissolved inorganic carbon and ammonia into the extracellular space; (b) Ca^{2+} ions in the microenvironment of the bacterium; (c) Ca^{2+} ions react with ions CO_3^{2-} to form calcium carbonate crystals (Siddique et al., 2011)

1.1.2 Metabolic conversion of nutrient

Alkaliphilic bacteria can precipitate carbonate either directly or indirectly. In direct calcite precipitation, bacteria metabolize calcium lactate and oxygen to produce calcium carbonate and carbon dioxide as a by-product (equation 1.3). Furthermore, in a carbonation phase (equation 1.4), carbon dioxide reacts with portlandite (calcium hydroxide), a result of cement hydration, to form calcium carbonate (Chaurasia et al., 2019;Wang et al., 2016). Bacteria act as a catalyst in this way, so a mineral precursor compound (calcium source) must be present in the matrix of the concrete specimens. However, there is a limit on how much mineral precursor compound can be added to the concrete mix (Farhadi and Ziadloo, 2020). This is because excessive precursors can have a detrimental effect on concrete properties, such as lowering final compressive strength (Jonkers et al., 2010). Below shows the biochemical reaction as aforementioned.

$$CaC_6H_{10}O_6 + 6O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O \quad (1.3)$$

$$CO_2 + Ca(OH)_2 \rightarrow CaCO_3 + H_2O \quad (1.4)$$

For indirect precipitation, alkaliphilic bacteria metabolize acetate (IUPAC naming: ethanoic acid) and oxygen into carbon dioxide (equation 1.5). In standard environment temperature and pressure, the carbon dioxide will be ionized into carbonate ion (equation 1.6). With the present of calcium ion, the carbonation occurred (Farhadi and Ziadloo, 2020). The possible equation of the reaction are as follows:

$$CH_3COO^- \ 2O_2 \rightarrow HCO_3^- + CO_2 + 2H_2O \quad (1.5)$$

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-} \quad (1.6)$$

$$2H^+ + CO_3^{2-} + 2OH^- + Ca^{2+} \rightarrow CaCO_3 \quad (1.7)$$

Alkaliphilic bacteria utilized the nutrient content to induced calcite precipitation. However, the characteristics of bacteria to be applied in the self-healing concrete need to withstand high alkaline conditions (due to the absence of urease as a neutralizing agent) and oxygen tolerant (Sangadji, 2017). Other than this, the bacteria must also able to prolong their survivability from the beginning till the mixing time as the precipitation process is much longer than urea hydrolysis (Sidiq et al., 2019). According to (Jiang et al., 2020), bacteria need to be in the form of spores instead of vegetative cells as it is more resistant to the alkaline environment and capable of withstanding high-speed shear force during mixing.

There is some limitation almost similar to the ureolytic calcite precipitation where the calcium salts might be forms and influence concrete serviceability. This is due to high demand of calcium source in the metallic conversion (Burbank et al., 2011), hence many researchers had did some intervention to tackle this issue.

1.2 Scope of the systematic review

As aforementioned, concrete self-healing is a wide range of studies. Importantly, the autonomous self-healing approach is more persuasive to be further discovered by most of the researchers due to the degree of “controllability” on the parameter of the concrete design. Development of self-healing concrete has begun for the past few decades; all the approaches are summarized in Figure 1.2. Although, there are many self-healing materials have been proposed recently, the majority of the researcher are more focused on microbial self-healing because it can be obtained from environmental sources such as the rhizosphere of plants, soil, or limestone (Hong et al., 2019). Therefore, in this review, only microbial self-healing concrete will be emphasized regardless of vascular or encapsulation methods. As to narrow down the scope, Bacillus species is chosen as the self-healing bacteria due to its high resistance characteristics to alkaline conditions (Van Tittelboom et al., 2010).

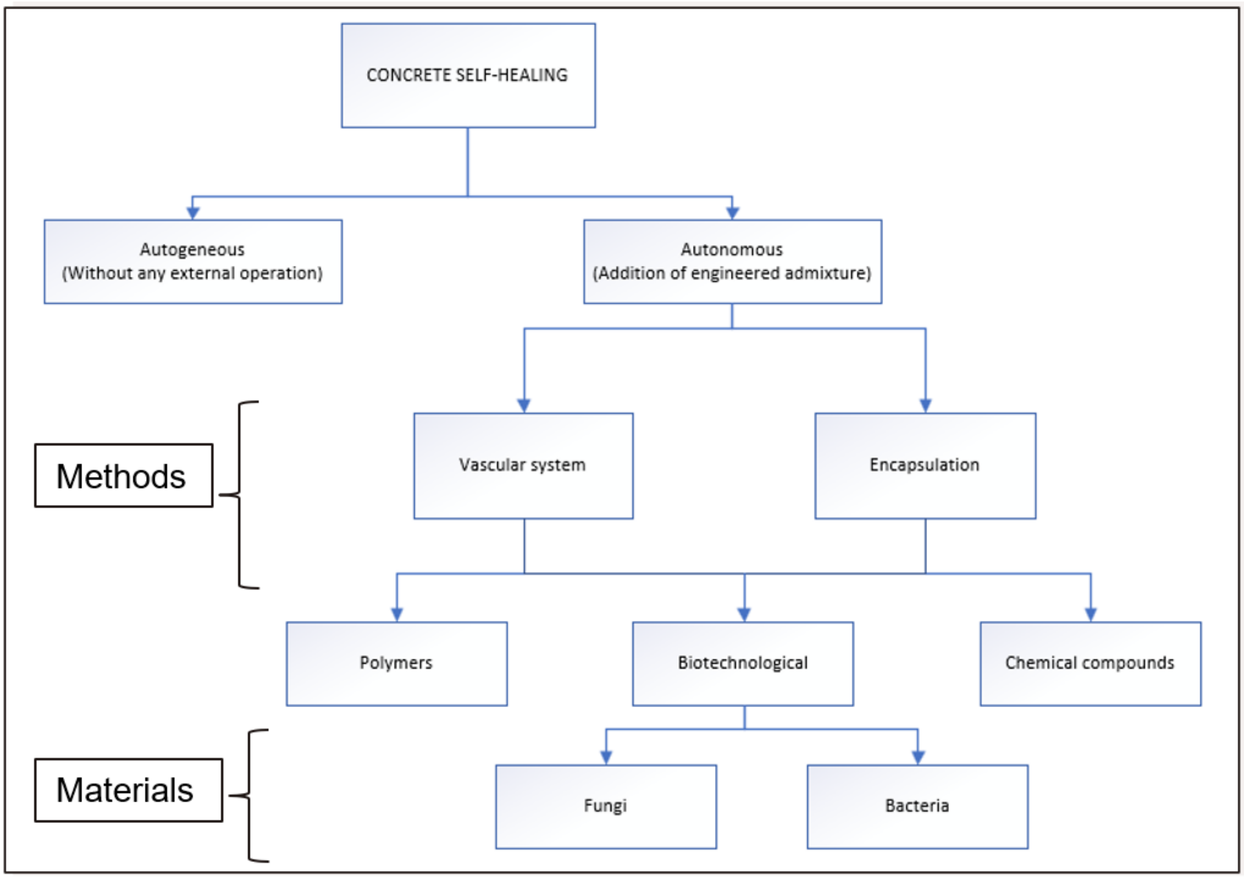

Figure 1.2 Classification of self-healing concrete modified from (Sidiq et al., 2019)

1.3 Review question

The review question was initiated by the researcher as described in sub-chapter 2.2.2. Microbial precipitation is always involved in bio cementation which is a complex process and yet to be further discovered due to its sensitivity to the environment such as temperature and nutrients (Reddy, 2013). The environmental factor possesses a vital role in self-healing techniques for concrete application due to the atmospheric exposure of concrete structure. All of these external factors could affect bacterial activity especially the production of calcium carbonate. Findings from several researchers, a bacterial activity could also be influenced by other internal factors such as dissolved inorganic carbon and calcium, nucleation side and pH (Hammes et al., 2002;Barton and Northup, 2011). Although optimization of bacterial activity is important in terms of its performance, the operational cost cannot be neglected for this application to be commercialized. It has been reported that the current studies of microbial self-healing are still unconvincing due to the offset caused by the extensive initial cost and time (Vaghari et al., 2015;Van Breugel, 2007). According to the analysis, the majority of operational cost contributions in this application are from the culture media (60% of the operational cost) (Kristiansen, 2001;Lee et al., 2018). Furthermore, the method to evaluate the performance of the healing capacity is crucial to identified as to address the method accurately and consistently in terms of its usefulness. To tackle this, offset effectively, this review will systematically analyze all the possible studies for the optimization of cultivation and sporulation only. The related issue mentioned above is converted into the review question as follows:

1) RQ 1: What are the factors that influence the performance of microbial (Bacillus species) self-healing?

2) RQ 2: What are the media required to create a conducive environment for cultivation and sporulation of Bacillus species in microbial self-healing?

3) RQ 3: What are the possible methods to evaluate the performance of cultivation and sporulation of bacillus species in microbial self-healing

1.4 Objectives of the systematic review

1) To determine the method of cultivation and sporulation of Bacillus species in self-healing concrete.

2) To identify the optimum condition for the cultivation and sporulation of Bacillus species in self-healing concrete.

1.5 Significance of the systematic review

The method of cultivation and sporulation of bacteria is an initial start of the self-healing concrete. The availability of resources and environmental conditions decide the method to grow the bacteria and a list of comparative methods can assist the application of the self-healing concrete to be more practical. Microbial self-healing is very dependent on either external factors or internal factors. This review will reveal the mechanism behind each factor and its recommendation to provide an optimum condition for cultivation and sporulation. In addition, it might be difficult to identify the essential cultivation and sporulation media and its content, this review provides a guideline in establishing a basic medium and some intervention that had been carried out by other researchers. Last but no least, the reader can obtain information about the evaluation of cultivation and sporulation based on the precipitation performance as to improve the efficiency and effectiveness of material used by the reader or other researcher in microbial self-healing concrete.

CHAPTER 2

SYSTEMATIC LITERATURE REVIEW (SLR): A METHODOLOGY

2.1 Introduction

In this chapter, the process of producing this paper will be discussed in detail from the planning, conducting, and reporting stages (Figure 2.1). During the planning stage, the researcher formulates the review question and develops a review protocol that fits the review question. After assessing and finalized the planning stage, the researcher conducts the SLR by schedule a search strategy, undergo a quality assessment based on each of the papers to be included, then extract the data required based on the review question and synthesis the data carefully and systematically. At the last stage, the researcher will report the findings from the literature review of those papers to be included (Xiao and Watson, 2019).

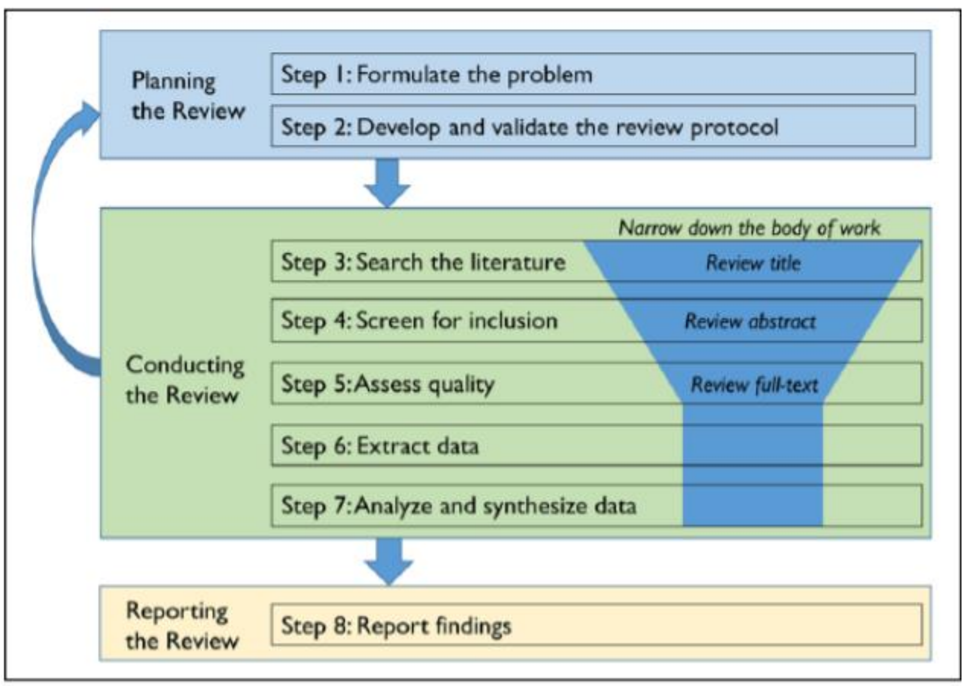

Figure 2.1 Process Of Literature Review (Xiao and Watson, 2019)

2.2 Planning of SLR

Formulation of review questions and development review protocol is the main component of structuring the SLR at the beginning stage. In the below subsection, the researcher will discuss the method of formulating the review question from the review objectives and also how it relates to the topic "Cultivation and Sporulation of Bacillus Species in Self-Healing Concrete: A Systematic Literature Review". Writing a plan of what item that the researcher wanted to include is very crucial in a systematic review. A plan often refers to review protocol in SLR where it describes various types of studies that will be located, evaluated, and synthesize. The purpose of the planning is to minimize the biased by developing a standard procedure when encountering any conflict during searching, extracting, and analyzing the result (Bettany-Saltikov, 2012). In this review, the protocol is according to the checklist as stated in the widely accepted Preferred Reporting Items for Systematic Review and Meta-analysis statement (PRISMA checklist 2009) (PRISMA, 2015a). There is some modification on the PRISMA checklist 2009 for fitting the purpose of this engineering systematic literature review.

2.2.1 Formulation of review question

The objective of the systematic literature review is to answer a clear and explicit research question by converting the gap of knowledge into an answerable and deeply constructed research question (University, Jan 28,2021). A well-formulated review or research question **to** ease the researcher for data extraction, data synthesis, and reporting during the inclusion and exclusion phase (Xiao and Watson, 2019). As mentioned in Chapter 1, there are several types of self-healing concrete, but only the bacterial self-healing concrete will be discussed in this paper. According to the PRISMA checklist 2009 item number 4, the explicit statement of the question is developed from the four major components which are participants, interventions, comparisons, outcomes, and study design (PICOS) (PRISMA, 2015a). However, the abovementioned element might not suitable for the engineering research systematic review as the initiative of the PRISMA was to assist the development of healthcare intervention (Liberati et al., 2009). Therefore, there are some alterations on the PRISMA checklist in developing the review question.

A broad research question may result in an unmanageable review, but this can be avoided by implement a more narrow review topic (Liberati et al., 2009). As mentioned earlier, research questions are developed from the gap of knowledge, in this paper timeline is narrowed to the latest 5 years (2016-2020) for studying the trend and insufficient research regarding the development of bacterial self-healing concrete. The researcher only uses one database (Scopus) for searching the related paper to review the trend and the gap of knowledge. To identify the relevant publications, a string was developed ("bacteria" AND "self-healing concrete") to search (access on November 26th,2020) within article title, abstract and keyword in Scopus, additionally some inclusion criteria is imposed as follows:

1) Written in English
2) Published between 2016 and 2020
3) Document type: article, conference paper, review
4) Source type: journal

All the result in the Scopus is extracted in the form of references (ris format) to Endnote as to ease the topic summarisation. There are about 116 publications throughout the five years with the abovementioned criteria. From the illustration graph in Figure 2.2, even though there is a small fluctuation between the years 2016 and 2018, it is considered an increasing trend for the number of papers with a related topic ("Bacteria" AND "Self-healing concrete") throughout these five years. All the papers are arranging according to the year of publication so that the development of self-healing is easier to trace. The researcher had gone through the abstract, objectives, conclusion of each paper to have a summary of a problem statement, objective, gap of study and suggestion. After that, all this information in every paper is summarised according to years as shown in Table 2.1. The mentioned subtopic and description in each year are almost varied, only a few topics are in common such as encapsulation. From Appendix 2 (compiled topic review), most of the researchers are shifting their direction on the effectiveness of self-healing by focusing on the microbial section instead of material-wise. There are some remarkable topics in the recent year 2020, for instance, survivability of bacteria, the optimum condition for self-healing bacteria, and optimization of Microbial Induced Calcite Precipitation (MICP). To identify the current trend of bacterial self-healing development in Malaysia, an interview (20th November 2020) with experts in self-healing concrete, Professor, Dr. Badorul Hisham Abu Bakar, from the Universiti Sains Malaysia is conducted. In the interview, Prof, Dr. Badorul mentioned that currently the local researcher facing common difficulties in producing self-healing bacteria, because there is no agency or authority especially to cultivate and sporulate those bacteria. Therefore, it is crucial to know more information about the sporulation and cultivation so that the researcher to practitioner can enhance the application of bacterial self-healing concrete into more practical. This issue is also mentioned in the two most citation papers (Vijay et al., 2017;Seifan et al., 2016a). Hence, this review addresses the following primary research question:

1) What are the factors that influence the performance of microbial (Bacillus species) self-healing?
2) What are the competent methods of cultivation and sporulation of bacillus species in different conditions for microbial self-healing?

3) What are the possible methods to evaluate the performance of cultivation and sporulation of bacillus species in microbial self-healing?

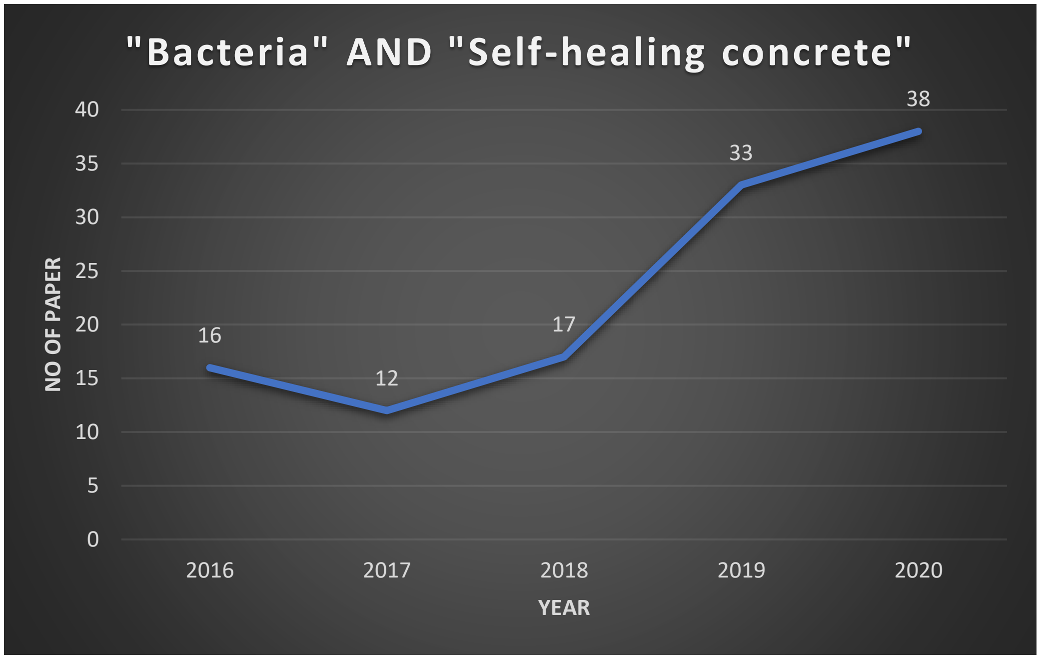

Figure 2.2 Number of Papers Throughout Five Year with Search String for Topic Review Purpose

Table 2.1 Review topic summary related to bacterial self-healing concrete

Year	Summary
2016	1**. Inhibition of strength** for the introduction of a microbial substance 2. **Encapsulation optimum condition** and method 3. **Performance** of microbial concrete to fill the crack
2017	1. **Optimisation of bacteria** such as survivability of bacteria, maximize the capabilities of bacteria by determining the factors (environment, types of bacteria, method
2018	1.Due to limitation especially of the **effectiveness of the encapsulation approach** and effectiveness of MICP, therefore some interventions are made such as **magnetic iron oxide nanoparticles, non-anoxic bio-granule, fungi**
2019	1.**Growth condition** of bacteria 2.The **type of bacteria used** (especially in optimizing Bacillus Subtilis 3.Type of **encapsulation**

2020	1.**Type of encapsulation** to ensure survivability and also **the cost of the self-healing material**. 2.Practical research such as is the **self-healing concrete be mixed in a bulk** 3.**Interfacial zone performance** in the microscopic observation

2.2.2 Review of protocol

Review protocol is a preset plan that reduces the researcher bias in conducting the review so that the quality of the SLR is enhanced (Xiao and Watson, 2019). This advanced plan before conducting the SLR preventing the researcher from changing the way of review the paper after the studies is identified (Bettany-Saltikov, 2012). Other than this, a review protocol increase the reliability of the review as other researcher or reader that interested in this paper can repeat the procedure to cross-check for validation (Xiao and Watson, 2019). Since the review protocol is well-organized, unique, and varies according to the review topic, usually the protocol will be registered under an international database called PROSPERO, but this applies to health research only (National Institute for Health Research 2021). Therefore, the researcher has by-pass this section even it has been mentioned in the PRISMA checklist 2009 (PRISMA, 2015a).

In this paper, the review protocol almost the same as the conventional SLR which describes all elements of the review including the objective of study, review question (already discussed in section 2.2.1), searching strategy, quality assessment criteria, method of data extraction, and synthesis (Gomersall et al., 2015;Gates, 2002). The components of the protocol are in sequence and illustrated in Figure 2.3. Moreover, each of these components will be discussed in details in each of the subchapters as follows:

- Identify review question (Chapter 2, section 2.2.1)
- Define search strategy (Chapter 2, section 2.3.1)
- Define quality criteria (Chapter 2, section 2.3.2)
- Define data extraction (Chapter 2, section 2.3.3)
- Define data synthesis (Chapter 2, section 2.3.3)

Figure 2.3 Components of SLR Protocol

2.3 Conducting of SLR

At this conducting stage, the researcher will discuss the searching strategy based on the objective of this review paper. The aims of the review paper are derived from the review question which was previously mentioned in section 2.2.1. Subsequently, the quality criteria will be defined to filter the inclusion paper in the search before data extraction and synthesis is carried out.

2.3.1 Systematic searching strategies

The search framework is according to the PRISMA flow diagram which consists of four steps (identification, screening, eligibility, and included) as shown in Figure 2.4 (PRISMA, 2015b). According to the PRISMA flow diagram, the initial search is made up of several databases, due to the access granted from the researcher's side, Scopus will be the only selected database to search. Although the final included article is sourced from one database, it does not influence the result of the search and this fulfills the criteria in PRISMA checklist 2009 (item no 8) (PRISMA, 2015a).

2.3.1(a) Identification

In other to develop a comprehensive search, the first phase of search strategy (identification) is subdivided into several steps as follows:

a) Write out the objective (related to the review question) and identify the keyword
b) Enrich the terms
c) Develop search string

In this paper, there are two objectives related to the review questions. Additionally, another statement that relates to the research question is created to increase the range of possibilities of the search. A keyword is identified based on the concept domain and other important elements (Kitchenham and Charters, 2007). The identification of the main terms is listed as follows:

A. Objective:

1. To determine the method of cultivation and sporulation of Bacillus species in self-healing concrete.
2. To identify the optimum condition for the cultivation and sporulation of Bacillus species in self-healing concrete.

B. Others

1. Biological behavior of Bacillus species in the application of self-healing concrete.

Table 2.2 Main terms derived from the 2 objectives and others

Derive the main terms (from O.1):	Derive the main terms (from O.2):	Others (1)
1. Method 2. Cultivation 3. Sporulation 4. Bacillus 5. Self-healing concrete	1. optimum 2. condition 3. cultivation 4. Bacillus 5. Self-healing concrete	1. Biological behaviour 2. Bacillus species 3. Application of direct method 4. Self-healing concrete

According to an article (Rowley and Slack, 2004), the search statement listed can be spread by synonyms, abbreviations, alternative spellings, and other related terms. Moreover, there are also other guidelines for enriching the keyword by free-text searching (also known as “natural language” or language use daily) (Lahlafi, 2007). The researcher uses both of these methods to generate comprehensive keywords to generate a search string using Boolean operators. The Boolean operators use as a link to combine the main terms and their respective synonym to further narrow down the topic (Brereton et al., 2007). The search string and its respective main terms are presented in the Table 2.3,2.4 and Table 2.5.

Table 2.3 Enrich the term and search string from Objective 1

Main term	Method	Cultivation	Sporulation	Bacillus	Self-healing Concrete
Enrich term	1. Technique 2.Way 3. Process 4. Approach 5.Procedure	1. Agriculture 2. Garden 3. Plant 4. Production	1.Spore Formation 2.Germination		1.concrete 2.crack concrete 3. Bio Concrete
Search string: ("method" OR "technique" OR "way” OR “process” OR “approach” OR “procedure”) AND ("cultivation” OR “agriculture” OR “garden” OR “plant" OR “production”) OR ("sporulation” OR “germination”) AND ("Bacillus”) AND (“self-healing concrete” OR “concrete” OR “crack concrete” OR “bio concrete”)					

Table 2.4 Enrich the term and search string from Objective 2

Main term	Optimum	condition	Cultivation	Bacillus	Self-healing Concrete
Enrich term	1.Best 2.Optimal 3.Good Situation 4. Ideal 5. Favourable 6.Favorable	1.Requirement 2.Environement	1. Farm 2. Agriculture 3. Garden 4.Plant 5. Production 6. Sporulation 7. Germination		1.concrete 2.crack concrete 3.Bio Concrete

			8. Formation		
Search string (“condition” OR “environment” OR “requirement”) AND ("cultivation" OR "agriculture" OR "garden" OR "plant” OR “production” OR "sporulation" OR” germination" OR “formation”) AND ("Bacillus”) AND (“self-healing concrete” OR “concrete” OR “crack concrete” OR “bio concrete”)					

The string above mentioned will be applied in the database (Scopus) and search within article abstract and keyword only. Results are illustrated in Figure 2.4 where the first search string based on objective 1 are 47 articles whereas the second search string has 41 articles and the “Others 1” search string obtains only 17 articles. All the information of these searches is recorded and compute in a table as shown in Table 2.6. This enables the researcher to retrace the literature search and to repeat the search on the database and source regularly to discover a new material that has surfaced since the initial search (Okoli and Schabram, 2010).

Table 2.5 Enrich the term and search string from Others 1

Main term	Biological	Behaviour	Bacillus	Self-healing Concrete
Enrich term		1.Behavior 2.Activities 3.Manners 4.Characteristics		1.concrete 2.crack concrete 3. Bio Concrete
Search string: (“biological”) AND (“behaviour” OR “behavior” OR “activities” OR “manners” OR “characteristics”) AND ("Bacillus ") AND (“self-healing concrete” OR “concrete” OR “bio concrete” OR “crack concrete”)				

Table 2.6 Search string information

Related statement	Search string applied in Scopus	Date of search

Objective 1	TITLE-ABS-KEY ((“method” OR “technique” OR “way” OR “process” OR “approach” OR “procedure”) AND (“cultivation” OR “agriculture” OR “garden” OR “plant” OR “production”) OR (“sporulation” OR “germination”) AND (“Bacillus”) AND (“self-healing concrete” OR “concrete” OR “crack concrete” OR “bio concrete”))	5th December 2020
Objective 2	TITLE-ABS-KEY ((“condition” OR “environment” OR “requirement”) AND (“cultivation” OR “agriculture” OR “garden” OR “plant” OR “production” OR “sporulation” OR “germination” OR “formation”) AND (“Bacillus”) AND (“concrete” OR “bio concrete”))	5th December 2020
Others 1	TITLE-ABS-KEY ((“biological”) AND (“behaviour” OR “behavior” OR “activities” OR “manners” OR “characteristics”) AND (“Bacillus “) AND (“self-healing concrete” OR “concrete” OR “bio concrete” OR “crack concrete”))	5th December 2020

2.3.1(b) Screening

The date range in the strings is restricted to the latest 5 years to observe the current trend and to avoid the scope of review from going too wide. Other than this, the document type is restricted to the article, conference paper, review, and all papers limited from the journal and English version only. After filter from the criteria above mentioned, only 23, 21, and 5 papers are left in the string related to Objective 1, Objective 2, and Others 1 respectively.

2.3.1(c) Eligibility and inclusion

The result from each search string after a filter is extracted out in the form of reference format, then transfer to EndNote X9 to identify the duplicates. With the aid of the function applicable in EndNote X9, 11 duplicates paper was detected and the left will undergo last screening to obtain the inclusion paper to be reviewed in this study. Inclusion and exclusion criteria often establish based on either the review question or objectives (Kitchenham and Charters, 2007). Any irrelevant studies will be excluded (Xiao and Watson, 2019). A book from (Torgerson, 2003), mentioned that the inclusion and exclusion criteria need to be very focused and applied stringently. There are two key steps recommended by (Jesson et al., 2011), can achieve the describe criteria as above mentioned. In the first steps, a researcher will read the title, abstract, and maybe the introduction and conclusion from the article to eliminate some misleading paper that obtains from the search engine. Subsequently, a researcher will identify whether there is key information required for the data extraction (discussed in section 1.3.3). All the irrelevant paper (Table 2.7) will be precluded including papers that cannot be accessed in full. There are about 11 paper left to be reviewed, but all of this paper needs to carry out quality assessment before it is finalized for extracted and synthesized.

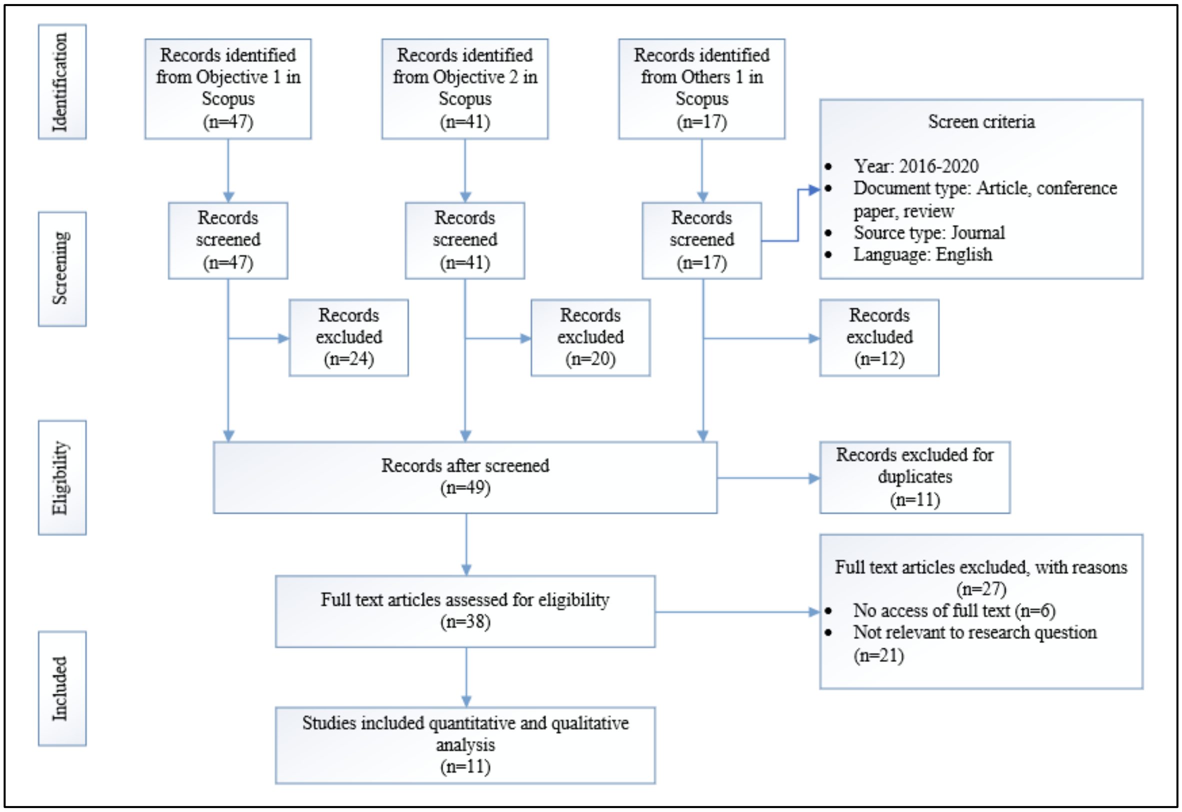

Figure 2.4: PRISMA flow diagram

Table 2.7 List of inclusion and exclusion paper with criteria

No	Year	Title	Description	Status	Remark
1	2016	Bioencapsulation of biosurfactant-producing bacillus subtilis (PTCC 1023) in alginate beads	not relevant to research question	excluded	bacteria carrier (imobilisation)
2	2016	Development of sustainable bacterial flyash concrete using the technique of microbiological induced calcite precipitation	not relevant to research question	excluded	cement replacement
3	2016	Properties of bacterial-based self-healing concrete - A review	no full text	excluded	-
4	2016	Utilization of carbon dioxide as an alternative to urea in biocementation		OK	
5	2016	Crack healing in concrete using various bio influenced self-healing techniques	not relevant to research question	excluded	bacteria carrier (imobilisation)
6	2016	Induced calcium carbonate precipitation using Bacillus species	no full text	excluded	-
7	2016	A cost effective cultivation medium for biocalcification of Bacillus pasteurii KCTC 3558 and its effect on cement cubes properties		OK	
8	2017	Biomineralization in metakaolin modified cement mortar to improve its strength with lowered cement content	not relevant to research question	excluded	cement replacement,biocementation
9	2017	Biologically active and volatile compounds in leaves and extracts of Nicotiana alata Link & otto from Bulgaria	not relevant to research question	excluded	extraction of bacteria
10	2017	New insights into the role of pH and aeration in the bacterial production of calcium carbonate (CaCO3)		OK	
11	2017	Alkaliphilic Bacillus species show potential application in concrete crack repair by virtue of rapid		OK	